

## Application Note

### ► High capacity sample cleaning for pesticide residue analysis using the example of different matrices

Category	Food analysis
Matrix	Olive oil, seawater sediment, mud
Method	GPC
Keywords	Cleanup, EuroClean, Bio-Beads
Analytes	
ID	VFD16, 12/10



#### Summary

Sample cleanup according to EN 12393, EN 1528, and US EPA SW-846 Method 3640A as well as other similar methods are time-consuming and solvent-intensive procedures. All these methods are based on a GPC separation with polystyrene gel in glass columns with diameters from 10 to 40 mm and lengths between 300 and 600 mm. With the widely used Bio-Beads SX-3 gel, a separation with dichloromethane or a mixture of cyclohexane/ethyl acetate takes about 45 minutes. Due to its different pore volume, the EuroClean GPC gel offers higher efficiency and separation performance according to the listed methods. Thus the column length can be reduced and the cleaning process can be carried out in only 30 min. Long-term validation of the EuroClean material has proven its robustness and applicability for sample cleaning of pesticide residues. Two typical and very different sample matrices were investigated and separated repeatedly in order to ensure suitability for environmental and food laboratories. With the significant reduction of GPC analysis run times, it is now possible to process two complete sequences of 15 samples each working day with the automated KNAUER GPC Cleanup Unit 6500. By using a shorter column bed length, solvent savings in the range of 30 % are possible.

#### Introduction

The GPC cleanup method for environment and food analysis is a powerful way to increase the limit of detection of multi-pesticide residues and other trace impurities. In the majority of cases the procedure is the same: the extraction step of a food or environmental sample is done with organic solvents. After separating the organic fractions, the high molecular weight compounds that would disturb the subsequent sub-trace analysis are removed via a GPC cleanup step. The GPC column packing materials typically have a molecular mass cut off of approx. 2 kDa. Since the impurities to be monitored only have molecular masses up to 400 Da, the undesired high molecular mass matrix elutes before the desired compounds.

## Experimental

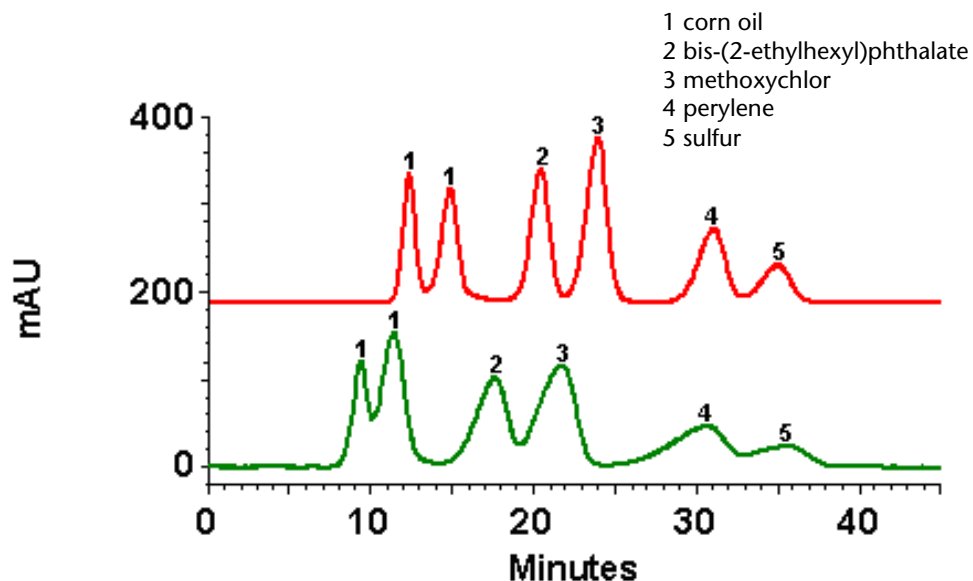
### GPC column preparation

Approx. 50 g of dry polystyrene-based GPC packing material are required to clean 5 to 10 ml of highly concentrated sample extracts. After swelling the packing material with an organic solvent such as THF, dichloromethane or ethyl acetate, the packing material is filled into a glass column of approx. 600 mm length and 25 mm inner diameter. Studies of long-term behaviour were done using a column inner diameter of only 9 mm to save solvent. The first column was filled with the widely-used and well documented Bio-Beads SX-3 gel.<sup>1-3</sup> Column performance was tested according to the US EPA 3640A method. The second column was filled with the new EuroClean GPC-material and tested in the same manner.

## Method parameters

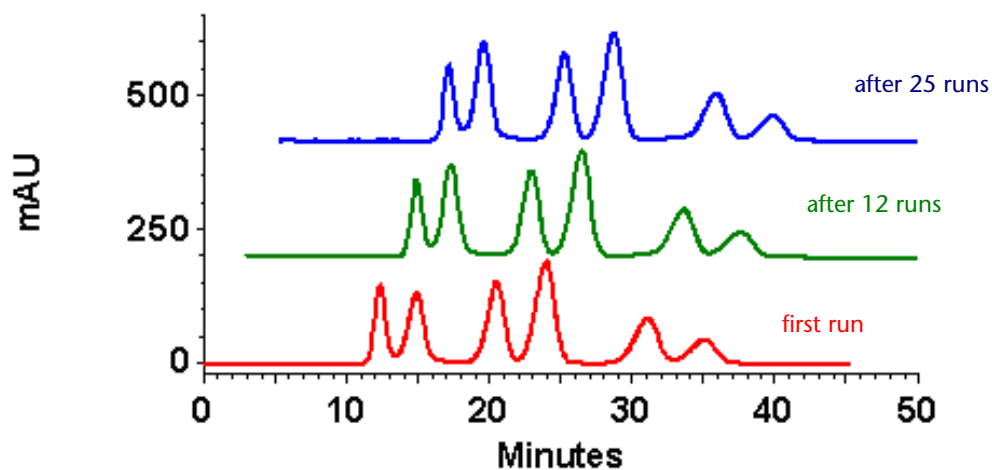
Column	glass column 700 x 9 mm
Packing material A	BioBeads SX-3, 600 mm bed length
Packing material B	EuroClean, 580 mm bed length
Eluent	cyclohexane/dichloromethane (85:15), dichloromethane
Flow rate	1 ml/min
Back pressure (Bio-Beads)	6 bar
Backpressure (EuroClean)	4 - 5 bar
Injection volume	200 µl
Temperature	ambient
Detection	UV at 254 nm (2 Hz); 3 mm flow cell
Analysis time	45 min

## Results

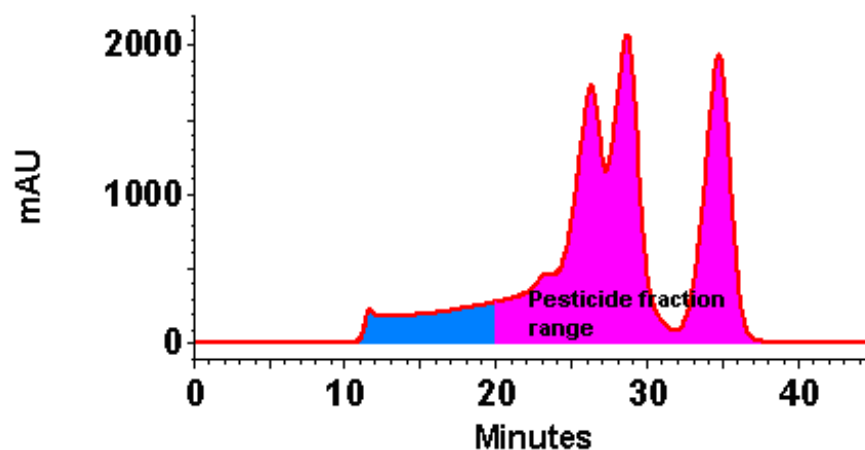


**Fig. 1**  
Standard test chromatogram with Bio-Beads (green) and EuroClean (red)  
Solvent: CH<sub>2</sub>Cl<sub>2</sub>

**Fig. 2**  
Long term validation with new EuroClean column using an environment sample extract according to EPA 3510C 1996 and EPA 3550C 2007  
Solvent: CH<sub>2</sub>Cl<sub>2</sub>



**Fig. 3**  
Sample cleaning of a mud extract according to EPA3550C 2007  
Solvent: CH<sub>2</sub>Cl<sub>2</sub>



The EuroClean column packing material showed a significantly higher resolution compared to the Bio-Beads column (see Fig. 1). This result offers the potential benefits to reduce column length, run time and solvent consumption. At first, we used an eluent mixture of cyclohexane/dichloromethane (85:15) and applied an olive oil sample. A rapid decrease of column performance was apparent after a few sample runs. We concluded that this eluent is not recommendable for EuroClean because the higher pore volume of this material is sensitive to nonpolar solvent compositions. Dichloromethane showed stable column performance under the conditions listed for olive oil samples according to US EPA SW-846 Method 3640A over 25 runs (see Fig. 2). Environmental samples from mud and sediment of seawater lagoons were tested according to EPA 3510C /EPA 3550C and showed a very stable separation performance over 25 runs (see Fig. 3).

## Conclusion

The EuroClean GPC sample cleanup packing material produces significantly higher resolution values for the separation of multi-pesticide residues in different matrices using dichloromethane as eluent. This implicates that with a reduced EuroClean column bed length, a dramatically shorter run time and less solvent consumption can be realized. During the validation procedure it became evident that nonpolar solvent compositions are not recommendable for this kind of sample cleaning.

## References

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## Recommended instrumentation

The GPC cleanup steps were performed using a KNAUER GPC Cleanup Unit 6500, equipped with Smartline Pump 1000 and 200 µl sample loops.

Description	Order No.
KNAUER GPC Cleanup Unit 6500	A5004V20
EuroClean GPC gel (100 g) prepacked glas columns	Y4012 upon request

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